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BEVERAGE

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The invention concerns a beverage of plant essences and extracts and other conventional ingredients that includes a combination of certain vitamins and colorless polyphenols.

Promotion of health is an function of beverages that for many people or in certain situations can be a deciding factor in drinking a beverage. This is important especially with those beverages that have a particular place as so-called health beverages, for example fruit juices and tea. The need for active promotion of health can have different degrees and thus covers a broad scale of desired grades of beverages. From just a generally health-promoting beverage all the way up to special medicine-like beverages there are many conceivable variations that are chiefly attractive due to the health motivation. On the other hand, the need for enjoyment is increasingly more important and the requirements on the flavor and aroma properties of beverages grow continuously higher (Uhr: Everything about Thirst [in German], Verlag Meininger 1980).

Many publications deal with the increase of chronic degenerative diseases like cardiovascular diseases and cancer. In the 35-65 year age group 31% of all deaths are due to cardiovascular diseases, and the fraction of those who die from cancer is about the same in this

age group. With increasing age there is a clear shift in the statistics concerning the causes of death. Beyond 65 years of age cardiovascular diseases take a dominant position, at more than 50%, whereas the fraction of those who die from cancer falls to about 20%. Based on the age pyramid, cardiovascular diseases become increasingly important, since the affected fraction among those over 70 years rises in the next 15 years from the current 12% of the general population to 20% and thus by nearly 70%. Since cardiovascular diseases are essentially nutritionally related, optimized nutrition takes on priority importance within the scope of preventing these diseases. The generally known risk factors like cholesterol, high blood pressure, overweight, diabetes and smoking are causative possibilities for about 50% of cardiovascular diseases, so that other causes must play a role for the remaining 50%.

Prominent importance here falls to the micronutrients, since of the 13 vitamins at least 6 are directly involved in the pathophysiological mechanisms of the development of arteriosclerosis.

It is known that the antioxidative vitamins C and E generally contribute to keeping the fraction of oxidized LDL cholesterol low, by which they prevent the development of arteriosclerosis. It is likewise known that three other vitamins participate in the prevention of cardiovascular diseases by lowering the level of homocystein in the blood.

These are the three B vitamins folic acid, vitamin B_6 and vitamin B_{12}. Since homocystein was recognized as an independent risk factor for development of arteriosclerosis, the supply of B vitamins merits increasing consideration, particularly since one can reduce the homocystein blood level through nutritive, i.e., relatively low doses of these B vitamins, and thus reduce the risk of cardiovascular diseases. According to the relevant publications, the contribution of homocystein to development of arteriosclerosis is due to its prooxidative effect and the adverse effect on blood clotting that goes along with this.

In the past there were often attempts to prove an isolated protective effect experimentally for some of these food ingredients. In the meanwhile it was shown that the protective effects cannot be achieved by individual substances.

Fruit juices are among beverages with high enjoyment and health value whose ingredients contribute particularly to physical well being. Besides their content of essential vitamins and minerals, digestion-promoting fruit acids and ballast substances, a number of other ingredients contained in small amounts in the fruits and the juices produced from them appear to be responsible for additional beneficial effects on the human body. A number of research results published in recent years contain indications that there are very complex relationships among these components, which are also called secondary plant ingredients or bioactive substances, and human metabolism. The so-called "French paradox" goes back to the effect of the secondary plant ingredients, for example, i.e., the fact that in spite of a relatively high-fat diet the rate of

cardiovascular diseases in France is relatively low. This is explained by the fact that with regular consumption of red wine protection against these diseases is produced by the secondary plant ingredients contained in the wine.

The polyphenols with clearly antioxidative potential that are responsible for the protective effect are phenolcarboxylic acids and their derivatives, whose importance for prevention of cardiovascular diseases has been described in many publications. Thus, an important role is attributed to the development of cardiovascular diseases the oxidation of LDL (low density lipoprotein) by oxygen radicals formed in the body (A. Rechner, C.-D. Patz, H. Dietrich: "Contribution to Evaluating the Antioxidative Capacity of Various Beverages" Flüss. Obst, 64, 1997, 62-65). Polyphenols, i.e., compounds belonging to various classes of substances like hydroxycinnamic acids, catechols and leucoanthocyanidines, anthocyanidines, flavanones, flavones and flavonols can reduce or prevent the oxidation of LDL by their antioxidative properties and the ability to trap harmful oxygen radicals and render them harmless. Besides the antioxidative effect a special protective effect on the vessel wall is ascribed to them. This long known protective effect of polyphenols has, for example, resulted in their use as cardiac drugs in the form of standardized plant extracts, where their antioxidative and vessel-protective effect is the focus of attention. Moreover, it is assumed that polyphenols have additional protective effects, but for these work toward a more detailed explanation has just begun.

The importance of the content of secondary plant ingredients in plant raw materials, especially in fruits, fruit juices, green and black tea and foods and beverages using these products has been treated in numerous publications. For example, a new category of foods and beverages has been sold under the designation "functional food" for some years especially in Japan and the USA, as well as in Europe, and these foods frequently contain secondary plant ingredients, especially in the form of green tea extracts, in addition to additives like ballasts, vitamins and minerals (C. Burke: "Functional Foods and Drinks, The Market in Japan," International Food Ingredients, No. 2 (1995), 39-42).

Some publications disclose beverages that contain certain polyphenols. For example, EP 0 416 667 A1 describes beverages with a stimulant effect, which are composed of at least 80 wt% water, at least 0.05% flavanols chosen from catechol, catechol derivatives, epicatechol, epicatechol derivatives and mixtures of these, and at least 0.2 wt% flavorings. The flavanols can come from green tea or other natural sources, where the ratio of caffeine to flavanols can be 1:1 to 1:30. The beverages can contain as other components fruit juice, sweeteners and carbon dioxide.

US 5,681,569 describes a liquid composition for improving cellular hydration and drinkability that includes 0.01-0.35% flavanols, 0.01-0.3% sodium ions, 0.005-0.08% potassium ions, 0.1-20% carbohydrates and water.

US 5,464,619 describes a composition, preferably in the form of a beverage, that increases cellular hydration and drinkability through a combination of selected amounts and types of electrolytes and carbohydrates. The liquid composition contains 0.01-0.35% flavanols, 0.01-0.3% sodium ions, 0.005-0.08% potassium ions, 0.05-10% fructose, 0.05-10% glucose, and water. The beverage can optionally contain 0.001-30% of a flavoring component and 0.01-0.04% caffeine or 0.05-0.09% chloride ions.

It is known that polyphenols and their primary oxidation products are among the most reactive compounds in fruits and vegetables. In the preparation and storage of beverages they are subject to changes that derive from enzymatic and nonenzymatic reactions among themselves and with other ingredients. Various reactions of the polyphenols lead to undesirable changes of color and are responsible, for example, for the occurrence of brownishness. Irreversible covalent bonds arise through condensation reactions, and the brown discoloration increases with an increasing degree of condensation and the solubility of these condensation products decreases. This can lead to instabilities and cloudiness.

Besides these undesirable brown discolorations and cloudiness caused by polyphenols in plant-based beverages, for example, tannin, tannin-protein, metal-tannin or starch-tannin cloudiness, polyphenols can also lead to other unpleasant sensory effects such as bitterness, astringency and the so-called "off flavor." For this reason in the production of juices in the fruit industry treatment and clarification processes are frequently used to remove the polyphenols and to avoid the changes caused by them.

However, according to the prior art there are still no beverages known that have, besides a high enjoyment value, a combination of bioactive ingredients of specific efficacy.

The task of this invention is therefore to make available stable beverages that equally have a high enjoyment value and pronounced protective effect and can make a contribution to protecting against oxidative stress. Moreover, another task of the invention is that the beverages have an ingredient density as high as possible, i.e., a content of bioactive substances that is as high as possible with respect to the energy value, while having energy value that is as low as possible.

It was surprisingly found that, besides the absolute magnitude of the antioxidative potential, the ratio of different polyphenols to each other in combination with nutritive doses of vitamins B_6, B_{12} and folic acid make an important contribution to the effect of the beverage in accordance with the invention. In particular the ratios of hydroxycinnamic acids and flavonol derivatives in each case to the sum of the colorless polyphenols play an important role for the nutritional value of the beverage in accordance with the invention.

The beverages in accordance with the invention are characterized, among other things, by a cardioprotective and collagen-stabilizing effect and with regular consumption a significant

decrease of the blood homocystein level can be observed. Due to their high ingredient density, i.e., their relatively low energy value with respect to the content of bioactive substances, they additionally have the advantage that their consumption does not lead to an undesirable high intake of calories and therewith possibly contribute to overweight, which must be seen as an independent risk factor for cardiovascular diseases.

The beverage of plant essences and/or extracts in accordance with the invention as well as optionally other conventional ingredients thus includes a combination of the following components:

- 0.8-30 mg/100 kcal, preferably 1.5-15 mg/100 kcal vitamin B_6
- 0.5-20 µg/100 kcal, preferably 0.8-10 µg/100 kcal vitamin B_{12}
- 0.1-6.5 mg/100 kcal, preferably 0.2-3.5 mg/100 kcal folic acid
- at least 10 mg/100 kcal, preferably 20-100 mg/100 kcal colorless polyphenols, and
- it has a TEAC value of 0.5-15,

and a ratio of the sum of the hydroxycinnamic acids, calculated as caffeic acid, to the sum of the colorless polyphenols from 1:1 to 1:20, preferably from 1:1.5 to 1:10, and a ratio of the sum of the flavonol derivatives, calculated as quercetin-3-glucoside, to the sum of the colorless polyphenols from 1:1 to 1:20, preferably from 1:1 to 1:10.

The said ratios are understood to be ratios by weight.

Preferably, the beverage in accordance with the invention contains 20-50 g/100 kcal, preferably 25-35 g/100 kcal total extract, where the sum of the dissolved components is understood as the total extract.

Likewise preferably, the beverage in accordance with the invention contains 1.5-6 g/100 kcal, preferably 2.5-4.5 g/100 kcal total acid, calculated as citric acid, and has a pH value of 3-4.5, preferably 3.5-4.3.

To achieve a balanced sweet-sour relationship the beverages in accordance with the invention can contain lemon juice, lemon juice concentrate and/or flavoring acids like citric acid, malic acid, lactic acid or tartaric acid, which can be used individually or in a mixture.

The beverage in accordance with the invention preferably contains 20-95 wt%, especially preferably 25-60 wt%, plant essences, extracts or other plant components.

The plant essences, extracts or other plant components that are used to make the drink are aqueous or alcohol essences from plants or in liquid or solid form, fruit juices or concentrates, fruit pulp or concentrate, fruit flavorings, vegetable juices or concentrates, vegetable pulp or concentrates, which can be used individually or in a mixture.

For example, the plant essences are apple juice concentrate, sloe juice concentrate, elderberry juice concentrate, quince juice concentrate, lemon juice concentrate and red grape

juice. Plant extracts and flavorings can be obtained, for example, from rose hips, hibiscus, apples, green tea, wild strawberry, elderberry flowers, mint, mango, banana, limes or lemons.

The beverage in accordance with the invention can contain, for example, water, sweeteners and vitamins as other conventional ingredients.

The sugars that are usually used in the beverage industry, for example sucrose, glucose, invert sugar syrup, glucose syrup, sugar substitutes like sorbitol, mannitol, as well as sweeteners like aspartame, acesulfam K, sucralose, saccharin, neohesperidine, cyclamate and thaumatin can be used as sweeteners in accordance with the invention. Beverages in accordance with the invention are preferably made without the addition of sugar in order to achieve an energy value that is as low as possible. The use of mixtures of the sweeteners aspartame and acesulfam K is especially suitable.

Beverages in accordance with the invention are enriched with vitamins of the B group, especially vitamins B_6, B_{12} and folic acid, in nutritionally relevant amounts. In addition, additives of other vitamins and provitamins, for example vitamins C and E, can also be contained.

The TEAC value (trolox equivalent antioxidative capacity) is understood to be the total antioxidative potential. To measure the TEAC value the synthetic water-soluble tocopherol "trolox" (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is used as reference substance.

The principle of the method is based on the delay of a linearly rising color reaction of metmyoglobin and ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) upon addition of hydrogen peroxide. The metmyoglobin acts as a catalyst and forms as the so-called ferryl myoglobin in the reaction with the resulting ABTS radical cation, a dye whose formation is inhibited in the presence of antioxidants.

The phenolic compounds or polyphenols that are summarized under the term hydroxycinnamic acids are monohydroxy and dihydroxy derivatives of cinnamic acid, especially the derivatives of caffeic acid, coumaric acid, chlorogenic acid, sinapic acid and ferulaic acid; the sum of the colorless polyphenols includes flavones, flavonols, flavanones, flavanols, procyanidines and dihydrochalcones, in addition to the hydroxycinnamic acids.

The colorless polyphenols are determined by separation by means of HPLC (high performance liquid chromatography) and detection of them at various wavelengths with a diode array detector.

The samples are processed in several steps:

· dimethylformamide (DMF) processing for samples that contain flavanone glucosides:

10 mL of the beverage in accordance with the invention is mixed with 10 mL dimethylformamide (DMF) and 10 mL of a freshly prepared solution of 3.55 g ammonium

oxalate in one liter of water. Then the mixture is heated for 10 min at 90°C on a water bath. The cooled solution is then filled to 50 mL with water.

· Methanol extraction of turbid juices, i.e., ones containing fruit pulp particles:

10 mL beverage is mixed with 20 mL methanol and vigorously stirred for 1 h.

· Solid phase extraction on a C18 phase:

The clear or clarified beverages are divided into a neutral and an acid fraction by solid phase extraction. For this the C18 phase (500 mg) is preconditioned with 20 bed volumes methanol and 20 bed volumes water each (pH 6) – neutral cartridge – or 0.5% acetic acid (pH 2) – acid cartridge. 2 mL beverage sample is now added to the neutral cartridge, washed with 5 mL water and the eluate together with the wash water, after adjusting the pH to 2 (with HCl), is quantitatively added to the acid cartridge. It is now washed with 5 mL 0.5% acetic acid. The polyphenols adsorbed on the cartridge are eluted with 5 mL methanol in each case. The eluates are evaporated at 35°C in a nitrogen stream. The residues are taken up in each case with 2 mL water/methanol 2/8, v/v.

· Fractionation on polyamide:

A glass column (250 x 27 mm) is packed to a packing height of 100 mm with polyamide (SC-6, Macherey-Nagel, Düren) preswollen in methanol. It is washed with water until methanol free. After inputting 20 mL juice it is then washed with 150 mL water. The neutral fraction is eluted with 300 mL methanol, and the acid fraction is eluted with 300 mL methanol/formic acid (98.5/1.5 w/w). The eluates are concentrated under mild conditions at 35°C and brought to a methanol content of about 20% with water.

· Cation exchange processing for separation of troublesome anthocyans:

2 mL cation exchanger (H^+ form/Dowex 50 WX4, 50-100 mesh) is packed into a small glass column. 10 mL clarified beverage or clear fraction is added. The first milliliter is discarded and only the remaining approximately 2/3 is reused.

The juices processed in this way are membrane filtered before the HPLC injection (0.2 µm filter).

HPLC conditions:

HPLC unit with diode array detector, gradient pump and column oven, column: LiChrospher RP18, 5 µm, 250 x 4.6 mm + 10 x 4 mm, precolumns with the same material, column temperature 40°C, initial pressure at start of gradient about 120 bar,

eluant A: 40mM formic acid in water,

eluant B: 40mM formic acid/methanol, flow rate: 1 mL/min.

Detection of the different groups of colorless phenolic compounds takes place at the following wavelengths:

Hydroxycinnamic acids:	325 nm
Flavones:	315 nm
Flavonols:	355 nm
Flavanones:	284 nm
Flavanols, procyanidines:	278 nm
Dihydrochalcones:	284 nm

The following examples serve for further illustration of the invention:

Example 1

To produce 10,000 L of a beverage in accordance with the invention 842 kg of a mixture of apple juice, sloeberry juice, elderberry juice and lemon juice concentrate, 0.9 kg of a mixture of aspartame and acesulfam K, 1 kg of a mixture of vitamins B_6, B_{12} and folic acid, 60 kg of a mixture of plant extracts and flavorings from rose hips, hibiscus, apple, green tea and wild strawberry, are brought up to 10,000 L with demineralized, degassed water. The degassed water has a residual oxygen content of <0.5, preferably <0.2 mg/L. Preparation takes place so that the resulting mixture is preferably heated to 50-55°C and degassed in a vacuum (for example, 120-150 mbar) while recycling the flavoring, where the flavoring condensate is continuously fed back to the product stream by the injector principle. After the subsequent brief heating needed for preservation (for example, 85-90°C, heating time about 10-15 sec) the hot beverage is sent directly to the filling machine, filled into suitable packages, for example bottles, and cooled to about 20°C in a recooler. Beverages in accordance with the invention can also be filled in cold sterilized form, i.e., the product which has been quickly heated at high temperatures for pasteurization is recooled in a heat exchanger to about 20°C and filled into sterile packages at this temperature.

The beverage prepared in this way has the following characteristics:

Total extract:	29.5 g/100 kcal
Total acid, calculated as citric acid	1.75 g/100 kcal
pH value	3.8
Vitamin B_6	2.7 mg/100 kcal
Vitamin B_{12}	0.98 µg/100 kcal
Folic acid	0.35 mg/100 kcal
TEAC value	1
Colorless polyphenols	34.5 mg/100 kcal
Fruit content	51 wt%
Hydroxycinnamic acids: colorless polyphenols	1:1.3
Flavonol derivatives: colorless polyphenols	1:7.4

Example 2

To produce another beverage in accordance with the invention 816.75 kg of a mixture of red grape juice and apple juice, elderberry juice and lemon juice concentrates, 36.25 kg of a mixture of plant flavorings and extracts from rose hips, elderberry flowers and apple, 0.45 kg of a mixture of aspartame and acesulfam K and 0.8 kg of a mixture of vitamins B_6, B_{12} and folic acid is brought up to 5000 L with demineralized and degassed water. The preparation and bottling take place as described in Example 1.

Example 3

432.3 kg of a mixture of lemon juice, quince juice and apple juice concentrate together with 18.475 kg of a mixture of flavorings and extracts from mint, mango, banana, limes, green tea, lemons and apples, 0.45 kg of a mixture of aspartame and acesulfam K and 1.0 kg of a mixture of vitamins B_6, B_{12} and folic cid are brought up to 5000 L with demineralized and degassed water. The mixture is prepared and bottled as described in Example 1.

Claims

1. A beverage from plant essences and/or extracts as well as optionally other conventional ingredients, where the beverage contains a combination of the following components:

0.8-30 mg/100 kcal vitamin B_6,

0.5-20 µg/100 kcal vitamin B_{12},

0.1-6.5 mg/100 kcal folic acid and

at least 10 mg/100 kcal colorless polyphenols, and the beverage

has a TEAC value of 0.5-15,

a ratio of the sum of the hydroxycinnamic acids, calculated as caffeic acid, to the sum of the colorless polyphenols from 1:1 to 1:20 and

a ratio of the sum of the flavonol derivatives, calculated as quercetin-3-glucoside, to the sum of the colorless polyphenols from 1:1 to 1:20.

2. A beverage as in Claim 1, which contains 20-50 g/100 kcal total extracts.

3. A beverage as in one of the preceding claims, which contains 1.5-6 g/100 kcal total acid, calculated as citric acid.

4. A beverage as in one of the preceding claims, which has a pH value of 3-4.5.

5. A beverage as in one of the preceding claims, which contains 20-95 wt% plant essences, extracts or other plant components.

6. A beverage as in one of the preceding claims, which contains water, vitamins and/or sweeteners as other components.

7. A beverage as in one of the preceding claims, which contains apple juice concentrate, sloeberry juice concentrate, elderberry juice concentrate, lemon juice concentrate, quince juice concentrate and/or red grape juice as plant essences.

8. A beverage as in one of the preceding claims, which contains a mixture of plant extracts and flavorings from rose hip, hibiscus, apple, green tea, wild strawberry, elderberry flowers, mint, mango, banana, lime and/or lemons.